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## DECLINE IN GLUCOSE-6-PHOSPHATASE ACTIVITY DURING PROLONGED POSTFIXATION WASHING

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In the course of a study on effect of glutaraldehyde fixation on glucose 6-phosphatase (G-6-Pase) activity, a chance observation showing that washing of fixed sections for longer periods of time resulted in considerable decrease in the enzyme activity was made.

Male DDD mice were anesthetized intraperitoneal injection of Nembutal. Trans-parenchymal perfusion of the liver was carried out using a 0.3 mm hypodermic needle and 5 cc syringe containing 2% glutaraldehyde buffered with cacodylate, 0.1 M, pH 7.0 at room temperature. The abdomen was opened to expose the liver and intestine. The perfusion needle was inserted into the parenchyma of the liver at the edge of the left lobe. A branch of the superior mesenteric vessels was cut open with scissors and the perfusion was started immediately at a flow rate of 0.25 ml per 5 seconds. The perfusion was continued for exactly 1.5 minutes. Following fixation, the tissue adjacent to the perfusion needle, about 1 cm in diameter, was quickly discarded. Small (0.1–0.2 g) blocks of the liver were immediately frozen on Dry Ice, weighed, cut at 30  $\mu$ , immersed and washed immediately in 0.3 M sucrose containing 0.01 M cacodylate (pH 7) at 4°C for 5, 15 or 30 minutes, and 1, 2, 4, 16 or 24 hours respectively. On the other hand, slices of the fixed livers, about 1 mm in thickness, were immediately immersed in the same washing solution for the same intervals of time and then cut at 30  $\mu$ . The volume of the washing solution was 100 ml per 0.1 g of tissues and the solution was changed three times, after 30 minutes, one hour and 2 hours. Both these sections and slices were immersed and shaken for the first 4 hours of the washing.

Lumps of the washed sections or fresh materials (0.1–0.2 g) were homogenized at 0–4°C in 10 volumes 0.25 M sucrose containing 0.01 M cacodylate (pH 7) in a Potter-Elvehjem type homogenizer for 5 minutes at 3000 rpm. G-6-Pase activity was assayed according to the method described by Leskes et al. (8). Incubation time was 20 minutes. The inorganic phosphate released was determined by the method of Allen (1). Proteins were estimated according to the method of Lowry et al. (9). The activity was expressed as  $\mu$  moles of phosphorus liberated per mg of protein per 20 minutes and the values for the washed tissues were put in relation to those obtained with 5 minutes washing.

The highest enzyme activity was present in sections washed for only 5 minutes and blocks washed for only 5 and 15 minutes (Table I). The activity decreased with the passage of washing time. In sections, the activity was reduced to about 70% of the value of 5 minutes washing after washing for 4 hours. In blocks, after 24 hours washing The activity reached

**Table I** G-6-Pase activity after immersion in sucrose solution following fixation

Intervals of immersion Specimens	min				hr				
	0	5	15	30	1	2	4	16	24
	% activity								
Sections	71.2 ±6.6	100	97.9 ±1.8	94.7 ±2.3	84.7 ±1.2	81.0 ±2.9	70.1 ±1.3	68.7 ±3.0	65.4 ±2.8
Blocks	72.4 ±7.6	100	99.8 ±1.3	98.4 ±1.1	94.2 ±1.2		80.6 ±0.7	74.4 ±0.8	70.1 ±2.1

Fixed sections (30 $\mu$ ) and tissue blocks (1 mm in thickness) were washed in 0.3M sucrose solution containing 0.01 M cacodylate, pH 7, at 4°C, homogenated and assayed for G-6-Pase. Fixation of tissues was carried out by transparenchymal perfusion with 2% glutaraldehyde for 1.5 min. Means for 5 measurements and standard deviation are given. Values are expressed relative to activities observed with specimens washed for 5 min.

just 70% of the value of washing for 5 minutes. It can be said that the enzyme activity reduced to 75% or less of the value of 5 minutes washing after washing overnight or more in both sections and tissue blocks.

Various periods of postfixation washing has been used for the cytochemical demonstration of G-6-Pase activity. For instance, fixed tissues were washed for 1 hour (4) (6) (7), 1–2 hours (2), 2 hours (11), 2–3 hours (5), 4 hours (3), overnight (12) or 24 hours (10). However, the present results indicate that postfixation washing for far shorter time than these is best for the demonstration of the enzyme activity.

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